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The Effects of Postexercise Feeding on Saliva Antimicrobial Proteins

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The purpose of this study was to determine the effects of a carbohydrate (CHO) and protein (PRO) drink consumed immediately after endurance exercise on saliva antimicrobial proteins known to be important for host defense. Eleven male runners ran for 2 hr at 75% $\text{VO}_{2\text{max}}$ on 2 occasions and immediately postexercise were provided, in randomized order, either a placebo solution (CON) or a CHO-PRO solution containing 1.2 g CHO/kg body mass (BM) and 0.4 g PRO/kg BM (CHO-PRO). The solutions were flavor and volume equivalent (12 ml/kg BM). Saliva flow rate, lysozyme, α -amylase, and secretory (S) IgA concentrations were determined from unstimulated saliva samples collected preexercise, immediately postexercise, and every 30 min until 180 min postexercise. CHO-PRO ingestion immediately postexercise resulted in a lower saliva flow rate than with CON at 30 and 60 min postexercise. Saliva lysozyme concentration increased immediately postexercise in both trials compared with preexercise ($p < .05$), and CHO-PRO ingestion immediately postexercise resulted in a higher saliva lysozyme concentration in the first hour of recovery than with CON (125% greater at 30 min, 94% greater at 60 min; $p < .01$). Saliva SIgA concentration decreased below preexercise concentrations 90–150 min postexercise ($p < .001$), with no effect of CHO-PRO. Saliva α -amylase activity was unaffected by exercise or CHO-PRO refeeding. CHO-PRO refeeding did not alter the secretion rates of any saliva variables during recovery. In conclusion, immediate refeeding with CHO-PRO evoked a greater saliva lysozyme concentration during the first hour of recovery after prolonged exercise than ingestion of placebo but had minimal impact on saliva α -amylase and SIgA responses.

Keywords: immune, mucosal, IgA, lysozyme, amylase

Athletes are advised to consume carbohydrate (CHO) and protein (PRO) immediately after prolonged strenuous exercise to help replenish muscle glycogen stores and aid growth and repair (Hawley, Burke, Phillips, & Spriet, 2011; Rodriguez, DiMarco, & Langley, 2009). As an additional benefit, this dietary strategy may also maintain immune function in the immediate postexercise period, when immune function is known to decrease; indeed, this period has often been described as an “open window” for susceptibility to upper respiratory illness (URI; Walsh, Gleeson, Shephard, et al., 2011). In line with this, we recently demonstrated that CHO-PRO feeding immediately after 2 hr of strenuous exercise prevented the decrease in neutrophil degranulation experienced when only water was consumed in the recovery period (Costa et al., 2009; Costa, Walters, Bilzon, & Walsh, 2011). Considering that most URI initiates at mucosal-epithelial surfaces, it is important to identify the impact

that prolonged strenuous exercise has on oral mucosal immunity during the recovery period and any potential favorable effects of a refeeding strategy.

The oral mucosal immune system comprises saliva flow and saliva secretions containing antimicrobial proteins (AMPs, e.g., immunoglobulin-A [IgA], lysozyme, α -amylase, lactoferrin, and defensins) with antibacterial and antiviral properties, which in combination form a first-line innate defensive barrier in the upper respiratory tract (Dowd, 1999; McNabb & Tomasi, 1981; Tenovuo, 1998; West, Pyne, Renshaw, & Cripps, 2006). Saliva flow provides a mechanical washing effect that recycles the oral mucosal surface composition. Saliva secretory (S) IgA is the most prevalent immunoglobulin in the oral mucosal immune pathway (Gleeson, 2000), which exerts its protective action by preventing pathogen adherence, excluding and clearing pathogens through immune complexes at the mucosal surface, and neutralizing intraepithelial viruses during viral epithelial transcytosis, thereby preventing further viral replication (Brandtzaeg et al., 1999; Mazanec, Nedrud, Kaetzel, & Lamm, 1993; Norderhaug, Johansen, Schjerven, & Brandtzaeg, 1999). The importance of saliva flow for optimal oral mucosal immunity is recognized in individuals suffering from dry-mouth syndrome (xerostomia), who present high

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incidence of URI (Fox, van der Ven, Sonies, Weiffenbach, & Baum, 1985). Low levels of saliva SIgA in nonathletic and athletic populations are related to increased self-reported URI (Gleeson, 2000; Hanson, Bjorkander, & Oxelius, 1983; Neville, Gleeson, & Folland, 2008). More recently, investigations have started to focus on the effects of exercise on important saliva AMPs other than SIgA alone, for example, lysozyme and α -amylase (Allgrove, Gomes, Hough, & Gleeson, 2008; Davison & Diment, 2010; West et al., 2010). These AMPs exert antimicrobial effects by disrupting microbe membranes, activating autolysins, and preventing bacterial adherence (West et al., 2006). We have previously demonstrated a limited effect of recovery feeding on postexercise saliva SIgA responses (Costa et al., 2009), but participants began exercise in a fasted state that does not represent normal practice, and we did not investigate the effects of recovery feeding on other salivary AMPs known to be important for host defense (e.g., lysozyme and α -amylase). Given, for example, that salivary α -amylase activity is increased after acute CHO ingestion (Hartmann, Brattinga, Van Kekem, Neyraud, & Dransfield, 2009), we might hypothesize that a CHO-PRO refeeding strategy would have a favorable effect on α -amylase during recovery from prolonged strenuous exercise. With this in mind, the aim of the current study was to investigate the effects of immediate CHO-PRO feeding on the saliva lysozyme, α -amylase, and SIgA responses in the 3-hr recovery period after strenuous prolonged exercise in trained runners.

Methods

Participants

Eleven healthy competitive endurance runners ($M \pm SD$ age 27 ± 7 years, body mass 77.1 ± 8.5 kg, height 1.79 ± 0.05 m, % body fat 15 ± 4 , VO_{2max} 62 ± 5 ml \cdot kg $^{-1} \cdot$ min $^{-1}$) volunteered to participate in the study. All participants were club-level road and mountain runners with an average of 10 ± 7 years competitive experience. They gave written informed consent before the study, which received approval from the local ethics committee. Participants were asked to complete a health and training log during their involvement in the study and in response to this reported no symptoms of infection or illness, nor were any medications taken in the 12 weeks before or during the study. Participants were also asked to refrain from alcohol and caffeine for 72 hr and exercise for 24 hr before preliminary testing sessions and each experimental trial. All volunteers were nonsmokers.

Preliminary Measurements

One week before the first experimental trial, and after a 24-hr period without training, the participants were asked to report to the laboratory, where height (stadiometer, Bodycare Ltd., Warwickshire, UK) and nude body mass (STW-150KE, Hampel Electronics, Zhonghe, Taiwan)

were recorded. Maximal oxygen uptake (VO_{2max} , Cortex Metalyser 3B, Biophysik, Leipzig, Germany) was estimated by means of a continuous incremental exercise test to volitional exhaustion on a motorized treadmill (HP cosmos Mercury 4.0, Nussdorf-Traunstein, Germany). From the VO_2 –work-rate relationship, the treadmill speed at 75% VO_{2max} was extrapolated and verified (11.4 ± 1.2 km/hr and 1% gradient).

Experimental Trials

During the 24 hr before the main experimental trials, participants were required to refrain from training. To control dietary intake, before each experimental trial a 24-hr diet was provided that catered for the participants' estimated energy requirements ($3,057 \pm 266$ kcal; Harris & Benedict, 1919) and provided 60% CHO, 23% fat, 17% PRO, and 35 ml/kg BM of water ($2,698 \pm 299$ ml; (Todorovic & Micklewright, 2004)). On two occasions separated by 1 week, participants reported to the laboratory at 7 a.m. after an overnight fast, where a standard breakfast (526 kcal; 118 g carbohydrate, 9 g protein, 2 g fat) was provided 2 hr before the onset of exercise. They were asked to empty their bladder and bowel before pre-exercise nude body-mass measurements. Urine specific gravity, determined by a handheld refractometer (Atago Uricon-Ne, NSG Precision Cells, New York, NY) was <1.020 g/ml in all participants on arrival at the laboratory for each trial, indicating euhydration (Armstrong et al., 1994). After preexercise saliva-sample collection, participants ran on a motorized treadmill for 2 hr at the previously determined treadmill speed that elicited 75% VO_{2max} . The 2-hr exercise bout was performed in ambient conditions of 20 °C and 59% relative humidity with two fans set at a speed of 2.3 m/s placed 1 m in front of the treadmill (VelociCal, TSI, St. Paul, MN). Heart rate and rating of perceived exertion (Borg, 1982) were recorded every 10 min during exercise. Participants were provided water at a rate of 3 ml \cdot kg BM $^{-1} \cdot$ hr $^{-1}$ (231 ± 26 ml/hr) during exercise and advised to drink the water ad libitum during the first 1 hr and 45 min of exercise. No water was ingested during the last 15 min of the exercise bout.

After the postexercise saliva-sample collections, nude body mass was recorded, and in randomized (cross-over) order participants performed one of two recovery interventions involving the same fluid volume (12 ml/kg BM, total volume 926 ± 103 ml), flavor (strawberry), and timing (immediately after exercise) of a recovery solution. The recovery solution consisted of either a placebo (0 g of CHO and PRO [CON]; Science in Sport, Blackburn, UK) or a CHO-PRO drink made to a 10% CHO solution that provided 1.2 g CHO/kg BM and 0.4 g PRO/kg BM (CHO-PRO; soya protein and maltodextrin modified Rego Recovery, Science in Sport, Blackburn, UK). Each participant received a further placebo bolus 1 hr postexercise in all trials (12 ml/kg BM, total volume 926 ± 103 ml) and remained in the laboratory for the entire 180-min recovery period, during which saliva samples were collected.

Saliva-Sample Collection and Analysis

Saliva samples were collected preexercise, immediately postexercise, and at 30-min intervals postexercise for 180 min. After thoroughly rinsing the mouth with water, participants were asked to swallow to empty the mouth before saliva was collected. Unstimulated whole saliva samples were collected by a dribbling method for 4 min into preweighed universal tubes (HR 120-EC, A & D Instruments, Tokyo, Japan). Participants were asked to lean forward and passively drool into the universal tube with minimal orofacial movements. Saliva volume was measured by weighing the universal tube immediately after collection to the nearest milligram. By assuming saliva density to be 1.00 g/ml, saliva flow rate in $\mu\text{L}/\text{min}$ was determined by dividing the volume of saliva by the collection time. Saliva was aliquotted into Eppendorfs and stored frozen at -80°C before analysis. After thawing, concentrations of saliva lysozyme (Biomedical Technologies, Stoughton, MA) and SIgA (DiaMetra, Segrate, Italy) were determined by ELISA, and α -amylase activity was determined by enzyme reaction assay kit (Salimetrics, State College, PA). A participant's samples were always assayed on the same plate. Secretion rates of saliva lysozyme, SIgA, and α -amylase were calculated by multiplying the saliva flow rate by the index concentration. The intra-assay coefficients of variation for saliva lysozyme, SIgA, and α -amylase concentration were 3.4%, 4.2%, and 3.6%, respectively.

Statistical Analysis

The data were examined using two-way (two trials, eight time points) fully repeated-measures ANOVA, except body-mass loss, which was assessed using a paired t test. All data were checked for normal distribution by calculating skewness and kurtosis coefficients. Where data violated the assumption of normality (positive skewness and kurtosis), data were log-transformed, and the transformed data were used for the ANOVA analysis. Assumptions

of homogeneity and sphericity were also checked, and where appropriate, adjustments to the degrees of freedom were made using the Greenhouse–Geisser correction method. Significant main effects were analyzed post hoc using Tukey's honestly significant difference test or Bonferroni-corrected t tests where appropriate. Data in text are presented as $M \pm SD$. For clarity, data in figures are presented as $M \pm SEM$. The required sample size was estimated to be 11 participants (www.dssresearch.com/toolkit/sscalc) using the mean values and standard deviations of previous data examining the effects of exercise stress and dietary intervention on oral mucosal immune indices (Allgrove et al., 2008; Costa et al., 2009). Alpha and beta levels were set at .05 and .8, respectively, both of which are standard estimates (Jones, Carley, & Harrison, 2003). The acceptance level of significance was set at $p < .05$.

Results

There were no significant Trial \times Time interactions for heart rate (exercise mean: 144 ± 45 beats/min) or rating of perceived exertion (exercise mean: 11 ± 4) and no significant difference in exercise-induced body-mass loss between trials (mean of trials: $2.4\% \pm 0.3\%$). For all the saliva variables, there were no between-trials differences before or immediately after the 2-hr exercise bout.

Saliva Flow Rate

A significant Trial \times Time interaction was observed for saliva flow rate, $F(7,70) = 2.7$ ($p < .05$; Figure 1). Compared with preexercise, saliva flow rate decreased significantly immediately postexercise on CON (-28%) and CHO-PRO (-28% ; both $p < .01$). During recovery, saliva flow rate increased significantly above preexercise from 90 min onward on CON ($p < .05$) and similarly from 90 min onward on CHO-PRO ($p < .05$). In addition, saliva flow rate was significantly lower on CHO-PRO at 30 min and 60 min than with CON ($p < .01$).

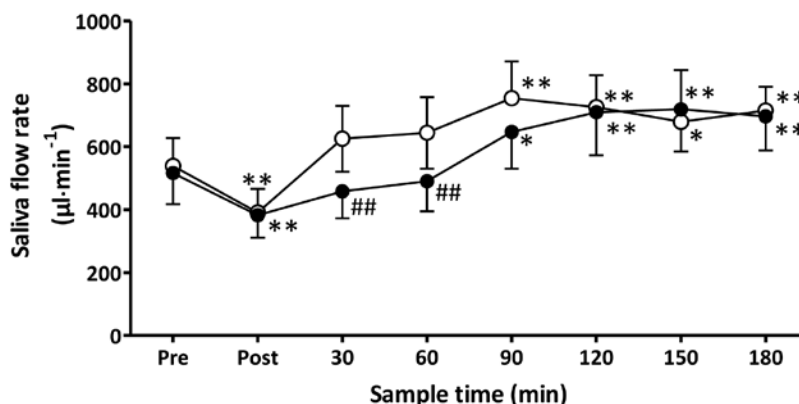


Figure 1 — Saliva flow-rate responses to placebo (open circles) and carbohydrate-protein (closed circles) feeding immediately after 2 hr running at 75% $\text{VO}_{2\text{max}}$. $M \pm \text{SEM}$ ($N = 11$). * $p < .05$ and ** $p < .01$ vs. preexercise. ### $p < .01$ vs. placebo.

Saliva Lysozyme Responses

Exercise and the provision of the CHO-PRO solution in the recovery period had a marked effect on saliva lysozyme concentration, interaction $F(7,70) = 3.3$ ($p < .01$; Figure 2A). Exercise resulted in a significant increase in the concentration of saliva lysozyme in both trials ($p < .05$). Saliva lysozyme concentration on CON returned to preexercise values at 30 min postexercise and onward during the recovery period. However, at 30 and 60 min during recovery, it was significantly higher ($p < .01$) on CHO-PRO than on CON. Although not significant, there was a trend for an interaction for saliva lysozyme secre-

tion rate, $F(7,70) = 1.9$ ($p = .078$), but no main effects were observed (Table 1).

Saliva α -Amylase Responses

Neither exercise nor postexercise refeeding had an effect on saliva α -amylase activity, $F(3.9,39.2) = 1.1$ ($p = .37$; Figure 2B), nor were there any main effects. No interaction was observed for saliva α -amylase secretion rate, although a significant main effect of time was detected, $F(2.2,22.1) = 3.5$ ($p < .05$), with a significant increase in saliva α -amylase secretion rate at 90 and 180 min compared with preexercise (Table 1).

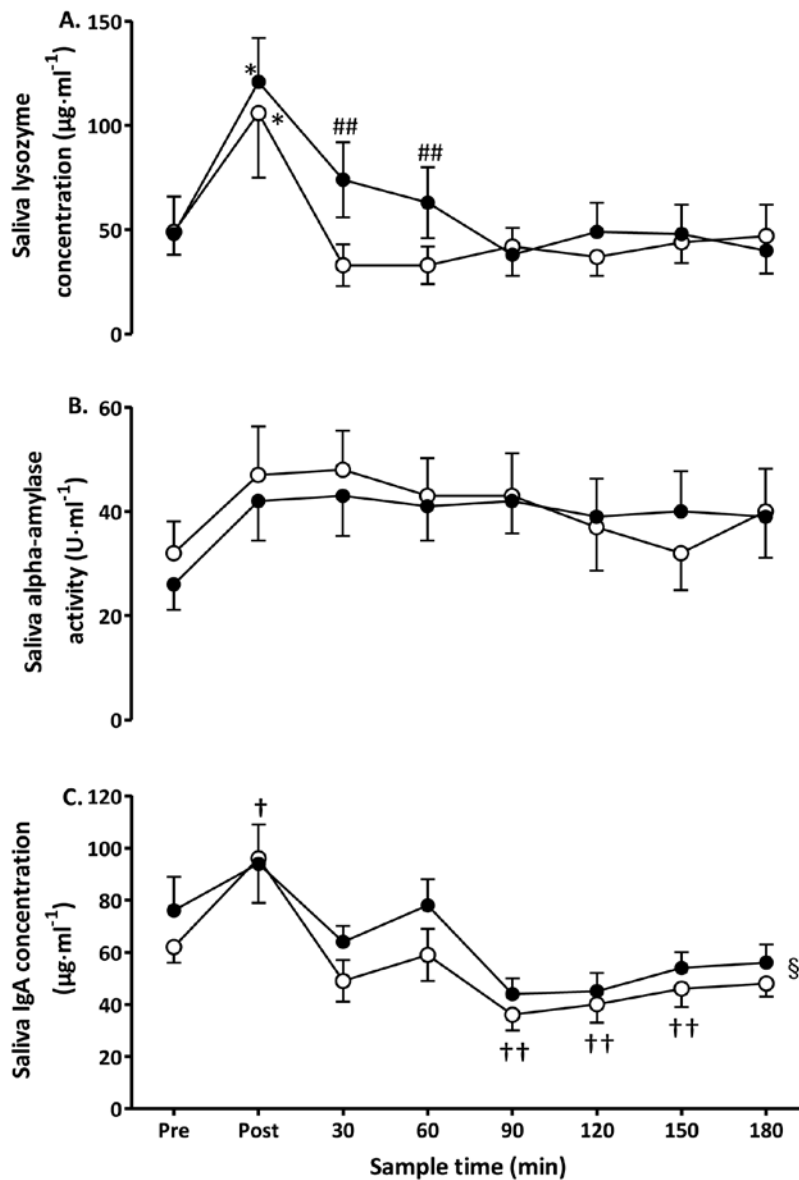


Figure 2 — (A) Saliva lysozyme concentration, (B) α -amylase concentration, and (C) SIgA concentration responses to placebo (CON; open circles) and carbohydrate-protein (CHO-PRO; closed circles) feeding immediately after 2 hr running at 75% $\text{VO}_{2\text{max}}$, $M \pm \text{SEM}$ ($N = 11$). * $p < .05$ vs. preexercise. ## $p < .01$ vs. CON. † $p < .05$, †† $p < .01$ main effect of time vs. preexercise. § $p < .05$ main effect of trial, CHO-PRO greater than CON.

Table 1 Saliva Lysozyme, α -Amylase, and SIgA Secretion-Rate Responses to Placebo (CON) and Carbohydrate-Protein (CHO-PRO) Feeding Immediately After 2 hr Running at 75% $\text{VO}_{2\text{max}}$, $M \pm SD$ ($N = 11$)

		Recovery Period (min)							
		Pre	Post	30	60	90	120	150	180
Saliva lysozyme ($\mu\text{g}/\text{min}$)	CON	32 \pm 60	49 \pm 99	16 \pm 12	20 \pm 20	29 \pm 26	27 \pm 25	26 \pm 16	29 \pm 26
	CHO-PRO	26 \pm 24	42 \pm 41	29 \pm 26	22 \pm 19	21 \pm 18	30 \pm 23	29 \pm 24	25 \pm 24
Saliva α -amylase (U/min)	CON	14 \pm 10	14 \pm 14	26 \pm 18	23 \pm 15	28 \pm 17	24 \pm 16	17 \pm 9	23 \pm 15
	CHO-PRO	11 \pm 9	14 \pm 11	17 \pm 20	15 \pm 13	21 \pm 13	21 \pm 11	21 \pm 10	22 \pm 12
Saliva SIgA ($\mu\text{g}/\text{min}$)	CON	31 \pm 18	34 \pm 28	25 \pm 12	33 \pm 21	22 \pm 10	26 \pm 18	27 \pm 13	31 \pm 14
	CHO-PRO	34 \pm 25	31 \pm 25	30 \pm 23	32 \pm 22	25 \pm 16	26 \pm 16	33 \pm 16	34 \pm 20

Note. Main effect of time is for saliva α -amylase only (90 min and 180 min greater than preexercise, $p < .05$).

Saliva SIgA Responses

The provision of the CHO-PRO solution immediately postexercise did not have any effect on saliva SIgA concentration—no interaction, $F(2.5,25.1) = 0.72$ ($p = .53$; Figure 2C)—although a main effect of trial, $F(1,10) = 6.3$ ($p < .05$, CHO-PRO greater than CON), and a main effect of time, $F(2.6,26.3) = 16.2$, ($p < .001$), were observed. Exercise increased the concentration of saliva SIgA by 37% ($p < .05$), while at 90, 120, and 150 min into the recovery period, it had decreased significantly compared with preexercise (-42% , -39% , and -28% , respectively, all $p < .01$). Saliva SIgA secretion rate was not affected by exercise or postexercise nutrition, no interaction: $F(7,70) = 0.25$ ($p = .97$; Table 1).

Discussion

The aim of the current study was to determine the influence of CHO-PRO feeding immediately after prolonged strenuous exercise on saliva AMPs known to be important for host defense. A novel finding was that CHO-PRO ingestion immediately after exercise evoked a greater saliva lysozyme concentration during the first hour of recovery than with CON; this occurred at a time when saliva lysozyme concentration tended to decrease below preexercise on CON. It remains to be shown if the greater saliva lysozyme concentration early during recovery with CHO-PRO ingestion is favorable for host defense. While CHO-PRO delayed the increase in saliva flow rate during the first hour of recovery compared with CON, the CHO-PRO refeeding strategy had minimal impact on saliva α -amylase and saliva SIgA responses.

The decrease in saliva flow rate immediately after exercise in both trials is likely due, at least in part, to the participants' experiencing modest dehydration during the 2-hr exercise bout, as reflected by a mean body-mass loss of 2.4% (Oliver, Laing, Wilson, Bilzon, & Walsh, 2008; Walsh, Gleeson, Pyne, et al., 2004; Walsh, Montague, Callow, & Rowlands, 2004). Similarly, the increase in saliva flow rate in both trials during the recovery period

likely reflects the provision of two large fluid boluses. However, the finding that saliva flow rate was lower in the first hour of recovery on the CHO-PRO trial than with CON is somewhat surprising, as it has previously been shown that CHO supplementation during and after prolonged exercise resulted in a higher saliva flow rate 1 hr after exercise than water alone (Bishop, Blannin, Armstrong, Rickman, & Gleeson, 2000). It is plausible that the nutrient-dense solution that our participants consumed on the CHO-PRO trial slowed the rate of gastric emptying (Sawka et al., 2007), thereby slowing the rate of rehydration, which was reflected by a lower saliva flow rate on CHO-PRO in the first hour of recovery. One other possibility is that stimulation of CHO receptors in the oral cavity influenced saliva flow rate, possibly via a central neural effect, but this requires substantiation.

CHO-PRO ingestion immediately after prolonged exercise resulted in a significantly higher saliva lysozyme concentration than with CON during the first hour of recovery, at a time when saliva lysozyme on CON tended to decrease below preexercise values. Since saliva flow rate was significantly lower on the CHO-PRO trial during the early stages of recovery, it is likely that this increase in saliva lysozyme concentration is a result of a concentrating effect. Indeed, in support of this contention, we did not observe any significant effects of exercise or refeeding on saliva lysozyme secretion rate (although there was a trend toward an interaction, $p = .078$). This demonstrates the importance of saliva flow rate in the regulation of oral mucosal immunity (Blannin et al., 1998; Oliver et al., 2007; Walsh et al., 1999). Nevertheless, the increased saliva lysozyme concentration after CHO-PRO refeeding, along with a tendency for saliva lysozyme secretion to be higher on the CHO-PRO trial than the CON trial at 30 min, may be considered favorable for the individual during the first hour of recovery, a period (often described as an open window) that has been previously reported to be associated with decreased oral mucosal immunity and increased reports of URI symptoms (Gleeson, 2000; Walsh, Gleeson, Shephard, et al., 2011).

Based on previous research showing that acute CHO ingestion can increase saliva α -amylase activity (Harthoorn et al., 2009), it was surprising that the provision of CHO-PRO during recovery did not augment the activity or secretion rate of saliva α -amylase compared with CON. However, an important finding of the current study was that neither saliva α -amylase activity nor secretion rate decreased below preexercise levels at any time point during recovery; indeed, saliva α -amylase secretion rate was higher than preexercise at 90 and 180 min of recovery. Although we did not conduct a trial without fluid intake during the recovery period, we surmise that provision of the large fluid bolus (independent of CHO-PRO content) immediately postexercise played a role in the maintenance of the salivary α -amylase response. As such, studies that investigate the effect of fluid intake and alterations in hydration status on the saliva α -amylase response are warranted. In the current study, the CHO-PRO was provided in a solution, but since mastication is known to increase saliva flow rate and the secretion of saliva α -amylase (Mackie & Pangborn, 1990), an interesting possibility that warrants investigation is whether providing a snack bar, in addition to fluids, during recovery may have a boosting effect on the salivary α -amylase response.

It is difficult to reach a consensus regarding the results of studies that have investigated the effects of acute, prolonged exercise on saliva SIgA. The discrepancies between studies are more than likely due to differences in exercise models, participant fitness, the degree of control over hydration status and nutritional intake, saliva-collection methods, and how SIgA is reported (as a concentration, as a ratio to protein or osmolality, or as a secretion rate; Blannin et al., 1998; Walsh et al., 1999; Walsh, Gleeson, Pyne, et al., 2011; Walsh, Gleeson, Shephard, et al., 2011). Unlike previous studies where participants were overnight fasted (Blannin et al., 1998; Costa et al., 2009; Walsh et al., 1999), here we examined the saliva SIgA response after endurance exercise in participants who had consumed a standardized breakfast 2 hr before; this is likely to better reflect normal practice. In this more realistic model, and in agreement with the previous investigation (Costa et al., 2009), our data demonstrate that CHO-PRO ingestion immediately after prolonged exercise does not alter the saliva SIgA response during recovery. It is important to highlight the discrepancies between studies regarding the effect of prolonged exercise on saliva SIgA responses. For example, here we show a decrease in saliva SIgA concentration during recovery from prolonged exercise that is similar to some studies (Costa et al., 2009; Gleeson et al., 1999; Gleeson et al., 2000) but not to studies showing an increase (Blannin et al., 1998; Laing et al., 2005) or no change (Bishop et al., 2000; Walsh et al., 1999). In addition, here we show no change in saliva SIgA secretion rate after prolonged exercise, which agrees with others (Costa et al., 2009; Walsh et al., 1999) but not those showing a decrease (Laing et al., 2005; Mackinnon & Hooper, 1994; Walsh, Bishop, Blackwell, Wierzbicki, & Montague, 2002)

or increase (Blannin et al., 1998). We propose that the decrease in saliva SIgA concentration during recovery in the current study and our previous refeeding work (Costa et al., 2009) reflects a dilution effect due to the increase in saliva flow rate observed when the additional placebo fluid bolus was provided 1 hr into recovery (Figure 1). Given the acknowledged influence of fluid intake (Bishop et al., 2000) and altered hydration status on saliva flow rate (Oliver et al., 2008; Walsh, Laing, et al., 2004; Walsh, Montague, et al., 2004), we hypothesize that the widely reported postexercise decrease in saliva SIgA concentration (Gleeson et al., 1999; Gleeson et al., 2000) and SIgA secretion rate (Laing et al., 2005; Mackinnon & Hooper, 1994; Walsh et al., 2002), which is also widely accepted to reflect a decrease in mucosal immunity, may, at least in part, reflect alterations in fluid balance. Appropriately designed studies are therefore required to test this hypothesis and unravel the effects of alterations in hydration status during exercise and recovery on saliva AMPs and host defense. Manipulating fluid balance in such studies will help us determine the degree to which the observed changes in saliva AMPs (e.g., SIgA) after acute bouts of prolonged exercise, and possibly during training, reflect an artifact of altered hydration status. Our results clearly support the argument that authors should express salivary AMPs as both a concentration and a secretion rate; indeed, the concentration and secretion rate of salivary AMPs responded differently to both the exercise stress and the recovery feeding regimen in the current study.

In conclusion, these findings demonstrate that immediate refeeding with CHO-PRO after prolonged exercise evoked a greater saliva lysozyme concentration during the first hour of recovery than did ingestion of placebo. However, immediate refeeding with CHO-PRO had no effect on saliva SIgA or saliva α -amylase responses. It remains to be shown whether the greater saliva lysozyme concentration early during recovery with CHO-PRO ingestion is favorable for host defense.

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